

CLAIMS

We claim:

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1. A method of detecting the presence of an intracellular analyte in one or more cells by flow cytometry, the method comprising:

- 5 a) fixing and permeabilizing said cells;
- b) catalyzing the deposition of tyramide in cells comprising said intracellular analyte;
- c) washing said cells in a medium comprising a chaotropic agent;
- d) contacting said cells with a detectable label that directly or indirectly binds
10 to tyramide, whereby cells comprising said intracellular analyte are specifically labeled;
 and
- e) detecting a signal from cells comprising said detectable label using a flow cytometric device, whereby said signal is at least 10-fold greater than a signal obtainable by standard flow cytometry methods.

15 2. A method of detecting the presence of an intracellular analyte in one or more cells by flow cytometry, the method comprising:

- a) fixing and permeabilizing said cells;
- b) catalyzing the deposition of tyramide conjugated to a detectable label in
cells comprising said intracellular analyte, whereby cells comprising said intracellular
20 analyte are specifically labeled; and
- c) washing said cells in a medium comprising a chaotropic agent;
- d) detecting a signal from cells comprising said detectable label using a flow cytometric device, whereby said signal is at least 10-fold greater than a signal obtainable by standard flow cytometry methods.

25 3. A method according to claim 1 or 2, wherein said signal is at least 20-fold greater than a signal obtainable by standard flow cytometry methods.

4. A method according to claim 1 or 2, wherein said signal is at least 50-fold greater than a signal obtainable by standard flow cytometry methods.

5. A method according to claim 1 or 2, wherein said catalyzing step comprises:

(i) incubating the fixed and permeabilized cells with a binding partner that specifically binds to said analyte, wherein said binding partner is conjugated to an enzyme capable of catalyzing the deposition of tyramide;

(ii) removing unbound binding partner from said cells; and

(iii) contacting bound binding partner with tyramide, whereby said enzyme catalyzes the deposition of tyramide in cells comprising said intracellular analyte.

6. A method according to claim 1 or 2, wherein said detectable label is a fluorochrome.

7. A method according to claim 6, wherein said fluorochrome comprises a fluorescent molecule selected from the group consisting of fluorescein, phycoerythrin, CY5, allophycocyanine, Texas Red, peridinin chlorophyll, and cyanine.

8. A method according to claim 5, wherein said enzyme is selected from the group consisting of hydrolases, peroxidases, oxidases, esterases, glycosidases and phosphatases.

9. A method according to claim 5 wherein said enzyme is horseradish peroxidase.

10. A method according to claim 1 or 2, wherein said catalyzing step comprises:

(i) incubating the fixed and permeabilized cells with a first binding partner that specifically binds to said analyte, and a second binding partner that specifically binds to said first binding partner, wherein said second binding partner comprises an enzyme, wherein said second binding partner is conjugated to an enzyme capable of catalyzing the deposition of tyramide;

(ii) removing unbound second binding partner from said cells; and

(iii) contacting bound second binding partner with tyramide, whereby said enzyme catalyzes the deposition of tyramide in cells comprising said intracellular analyte.

11. A method according to claim 10, wherein said second binding partner is an immunoglobulin-enzyme conjugate.

5 12. A method according to claim 1 or 2, wherein said one or more cells are one or more mammalian cells.

13. A method according to claim 12, wherein said one or more mammalian cells are selected from the group consisting of basal cells, epithelial cells, erythrocytes, platelets, lymphocytes, T-cells, B-cells, natural killer cells, granulocytes, monocytes, mast cells,
10 Jurkat cells, neurocytes, neuroblasts, cytomegalic cells, dendritic cells, macrophages, blastomeres, endothelial cells, HeLa cells, tumor cells, interstitial cells, Kupffer cells, Langerhans' cells, Langhans cells, littoral cells, tissue cells, adipose cells, CHO cells, KFL9, and K562 cells.

14. A method according to claim 1 or 2, wherein said one or more cells are cultured
15 cells.

15. A method according to claim 1 or 2, wherein said intracellular analyte is selected from the group consisting of intracellular cytokines, antigens, viral antigens, nuclear antigens, cytoplasmic antigens, organellar antigens, enzymes, cytoskeletal molecules, glycolipids, lipids, glycans, chaperones, RNA, DNA, messenger RNA, ribosomal RNA,
20 signal transduction proteins, and structural proteins.

16. A method according to claim 1 or 2, wherein said intracellular analyte is not a natural component of said one or more cells.

ai 17. A method according to claim 1 or 2, wherein said intracellular analyte cannot be detected by standard flow cytometry methods.

25 18. A method according to claim 1 or 2, wherein said one or more cells are obtained from a patient.

19. A method according to claim 18, wherein said signal is correlated to a diagnosis of a disease in said patient.

20. A kit for performing a method according to claims 1 or 2.

21. A method of detecting the presence of a target nucleic acid molecule in one or more cells by flow cytometry, the method comprising:

- a) fixing and permeabilizing said cells;
- b) contacting said cells with a probe nucleic acid molecule that specifically binds to said target nucleic acid molecule, wherein said probe nucleic acid molecule is conjugated to a first label, and a binding partner that specifically binds said first label, wherein said binding partner is conjugated to an enzyme capable of catalyzing the deposition of tyramide;
- c) contacting said cells with tyramide conjugated to a second label and a substrate for said enzyme, whereby tyramide is deposited in cells comprising said target nucleic acid molecule;
- d) washing said cells in a medium comprising a chaotropic agent; and
- e) detecting a signal from cells comprising said second label using a flow cytometric device.

22. A method according to claim 21, wherein said probe nucleic acid molecule is selected from the group consisting of a DNA molecule, an RNA molecule, a cDNA molecule, an aptamer, and a peptide nucleic acid (PNA) molecule.

23. A method according to claim 21, wherein said signal is at least 20-fold greater than a signal obtainable by standard flow cytometry methods.

24. A method according to claim 21, wherein said signal is at least 50-fold greater than a signal obtainable by standard flow cytometry methods.

25. A method according to claim 21, wherein said first label is a hapten selected from the group consisting of biotin, FITC, digoxigenin, trinitrophenol, and dinitrophenol.

26. A method according to claim 21, wherein said second label is a fluorochrome.
27. A method according to claim 26, wherein said fluorochrome comprises a fluorescent molecule selected from the group consisting of fluorescein, phycoerythrin, CY5, allophycocyanine, Texas Red, peridenin chlorophyll, and cyanine.
- 5 28. A method according to claim 21, wherein said enzyme is selected from the group consisting of hydrolases, peroxidases, oxidases, esterases, glycosidases and phosphatases.
29. A method according to claim 28 wherein said enzyme is horseradish peroxidase.
30. A method according to claim 21, wherein said binding partner is an
10 immunoglobulin.
31. A method according to claim 21, wherein said one or more cells are one or more mammalian cells.
32. A method according to claim 31, wherein said one or more mammalian cells are selected from the group consisting of basal cells, epithelial cells, erythrocytes, platelets,
15 lymphocytes, T-cells, B-cells, natural killer cells, granulocytes, monocytes, mast cells, Jurkat cells, neurocytes, neuroblasts, cytomegalic cells, dendritic cells, macrophages, blastomeres, endothelial cells, HeLa cells, tumor cells, interstitial cells, Kupffer cells, Langerhans' cells, Langhans cells, littoral cells, tissue cells, adipose cells, CHO cells, KFL9, and K562 cells.
- 20 33. A method according to claim 21, wherein said one or more cells are cultured cells.
34. A method according to claim 21, wherein said target nucleic acid molecule is not a natural component of said one or more cells.
35. A method according to claim 21, wherein said target nucleic acid molecule cannot be detected by standard flow cytometry methods.

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36. A method according to claim 35, wherein said signal is correlated to a diagnosis of a disease in said patient.

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